Extended spectrum β-lactamase-producing *Escherichia coli* faecal carriage in Spanish travellers returning from tropical and subtropical countries

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Abstract

The aim of this study was to investigate the prevalence of extended-spectrum β-lactamase (ESBL) -producing *Escherichia coli* in stool samples from 457 patients with travellers' diarrhoea who had travelled to tropical and subtropical countries. Ninety-seven ESBL-producing *E. coli* strains were isolated from 17.9% of the patients (82/457). CTX-M-15 was the most prevalent enzyme (80%) and India was the most visited country and showed the highest prevalence of positive samples (37.4%).

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Extended-spectrum β-lactamase (ESBL) production is the most common mechanism of bacterial resistance to third-generation cephalosporins. The ESBL-producing *Enterobacteriaceae* have become an endemic problem worldwide during the last decade, the CTX-M family being the most widespread. International travel has been characterized as a risk factor for both colonization and infection with extended-spectrum cephalosporin-resistant as well as carbapenem-resistant isolates [1–3], such as those carrying NDM-1 [4]. Many studies have focused on the prevalence of faecal carriers within specific countries, which, in 2007, was between 6.6% and 8.2% in Spain and was most associated with the CTX-M-14 enzyme [5,6]. However, studies addressing the faecal carriage of ESBL-producing *Enterobacteriaceae* in travellers are scarce [3,7–9]. The aim of this study was to investigate the prevalence of ESBL-producing *Escherichia coli* in stool samples from patients with travellers' diarrhoea who had travelled to tropical and subtropical countries.

Travellers with travellers' diarrhoea attending the Tropical Medicine Unit of the Hospital Clinic of Barcelona from March 2009 to July 2011 were analysed (n = 420). Travellers' diarrhoea was defined according to previously reported criteria [10]. Additionally, travellers with travellers' diarrhoea who attended the Emergency Department of the Hospital Clinic during the same period were also included (n = 37). In all cases, a standardized questionnaire covering clinical and epidemiological data was completed. All patients were followed until complete resolution of the symptoms.

Stool samples were screened for cephalosporin-resistant *Enterobacteriaceae* using chromogenic selective media (ChromID ESBL, bioMérieux, Marcy l'Etoile, France). The chromogenic plates used were inoculated as soon as the sample arrived at the laboratory in parallel with the inoculation of other conventional media for stool analysis. If all of the growing colonies had the same morphology and colour, only one colony was selected, otherwise the different colonies observed were picked up. The bacteria were identified by matrix-assisted laser desorption ionization–time of flight mass spectroscopy, and only *E. coli* isolates were selected. ESBL production was further confirmed using the double-disc synergy test (ceftazidime and cefotaxime with amoxicillin/clavulanic acid). Genes encoding ESBL (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) were detected by PCR amplification and sequence analysis using previously described conditions [11–13]. The ESBL-producing *E. coli* isolates were classified into the phylogenetic groups A, B_1, B_2 and D by multiplex PCR as previously reported [14]. The disc-diffusion method was performed for β-lactams, ciprofloxacin, gentamicin and cotrimoxazole and interpreted applying the EUCAST breakpoints published in 2011 (document V1.3 2011 http://www.eucast.org/antimicrobial_susceptibility_testing/previous_versions_of_tables/). Risk factor (travel destination) for acquisition of ESBL-producing *E. coli* was calculated using a logistic regression model. Categorical data were compared using the Fisher's exact test. All statistical analyses were performed using STATA (Stata Statistical Software) version 13.1 (StataCorp., College Station, TX, USA). The level of significance was established at the 0.05 level.

A total of 457 patients with travellers' diarrhoea were screened (268 women (58.6%) and 189 men (41.4%)): the overall median age being 33 years (interquartile range 28–40). The median trip duration was 20 days (interquartile range 14–31; $n = 433$). Eighty-two patients (17.9%) were colonized with 97 ESBL-producing *E. coli* isolates: 13 patients had two isolates and one patient had three isolates (Table 1). Among the 100 ESBL-encoding genes detected, the CTX-M family showed the highest prevalence (91%): 87.9% were identified as CTX-M-15, 6.6% as CTX-M-14, 3.3% as CTX-M-27 and 2.2% as others. The remaining nine genes (9%) belonged to the SHV type: 55.6% corresponded to SHV-12, 33.3% SHV-5 and 11.1% SHV-27. In total, three isolates showed the presence of two ESBL-encoding genes (Table 2). Statistical analysis showed a significantly increased percentage of acquiring a CTX-M group one enzyme when returning from Asia (p 0.0003). In addition, a high percentage (43.3%) of the cephalosporin-resistant isolates also produced TEM-1.

Several studies performed in Sweden and Canada have provided evidence that international travel is a risk factor for ESBL carriage, raising the prevalence from 2.4–4% in non-travellers to 23–36% in international travellers [7–9]. Similarly, two studies published in our country reported rates of 6.6% and 8.2% of ESBL colonization in non-travellers [5,6]. CTX-M-15 is highly disseminated in most parts of the world whereas CTX-M-14 predominates in Spain [5,15]. Although ESBL colonization of patients with TD was not assessed before foreign travel, according to these data it seems reasonable to suggest that international travel is also a risk factor among the Spanish population.

TABLE 1. Prevalence and risk factor for extended-spectrum β-lactamase-producing *Escherichia coli* by geographical distribution

Areas and countries	Number of patients (%)	Colonized patients (%)	Odds ratio[b]	(95% CI)	p-value
North Africa	40 (8.8)	5 (12.5)	0.81	0.31–2.07	<0.0001
Egypt	13	2			
Morocco	21	3			
East Africa	40 (8.8)	0	1.28	–	
West Africa	63 (13.8)	10 (15.9)	0.89	(0.40–1.98)	
Burkina Faso	7	1			
Ghana	4	2			
Mali	10	2			
Nigeria	2	1			
Senegal	23	4			
Central Africa	14 (3.1)	3 (21.4)	1.28	(0.55–2.99)	
Cameroon	6	1			
Congo	2	1			
Equatorial Guinea	2	1			
South Africa	9 (2)	2 (22.2)	1.34	(0.30–6.11)	
Mozambique	7	2			
Central America	41 (9)	4 (9.7)	0.64	(0.24–1.72)	
Guatemala	11	2			
Mexico	14	2			
South America	43 (9.4)	4 (9.3)	0.48	(0.17–1.40)	
Peru	13	3			
Venezuela	2	1			
Caribbean	21 (4.6)	0	1.28	–	
Eastern Asia	3 (0.6)	0	1.28	–	
South Asia	142 (31.1)	51 (35.9)	3.31	(1.82–6.01)	
India	131	49			
Nepal	9	2			
South East Asia	40 (8.8)	3 (7.5)	0.38	(0.12–1.25)	
Indonesia	8	1			
Malaysia	4	1			
Myanmar	1	1			
Thailand	16	0			
More than one region[a]	1 (0.2)	0	1.28	–	
Total	457	82 (17.9)			

[a]South Africa and South Asia.
[b]Odds ratio for deviations from the grand mean.

Antimicrobial susceptibility testing showed that all isolates were resistant to penicillins, second-, third- and fourth-generation cephalosporins and monobactams. However, in the presence of β-lactamase inhibitors, the levels of resistance decreased: 53.6% of the isolates were resistant to amoxicillin–clavulanic acid whereas only 3% of the isolates were resistant to piperacillin–tazobactam. All isolates were susceptible to carbapenems, except one that additionally harboured an NDM-1 carbapenemase, as previously reported by our group [4]. High percentages of resistance were also seen for other antimicrobials: 49.5% of the isolates were resistant to gentamicin, 79.4% to ciprofloxacin and 84.5% to cotrimoxazole. Concerning the phylogenetic group, 58.8% of the isolates belonged to phylogenetic groups A and B1 (52.6% and 6.2%, respectively), and 41.2% to the potentially virulent phylogenetic groups D (26.8%) and B2 (14.4%).

Travel destination is likely to be a major factor for ESBL-carriage positivity of faecal samples. Patients returning from South Asia (particularly India and Thailand), and North Africa (Egypt) are those usually most predisposed to become carriers [7–9]. In this study, most of the patients (40.5%) had travelled to the Asian continent, followed by 34.4% who had visited Africa and lastly, 18.4% who had travelled to Central and South America. The prevalence of ESBL faecal carriers was the highest for those patients visiting Asia (29.2%), particularly South Asia (Table 1), whereas lower values were obtained for Africa (12.7%) and Central and South America (9.5%). By far, India was the most visited country (131 patients) and showed the highest prevalence (37.4%). However, none of the 16 patients returning from Thailand was positive for ESBL carriage. For the African continent, Senegal, Morocco and Egypt were the most visited countries with prevalences of 17.4%, 14.3% and 15.4%, respectively. Lastly, Mexico, Peru and Guatemala, with respective prevalences of 14.3%, 18.2% and 23.1%, were the most visited destinations in Central and South America. Comparison with endemic values is difficult because studies reporting the prevalences in tropical and subtropical countries are scarce. Nonetheless, in India the prevalence of such isolates obtained from any type of clinical specimen has been assessed and ranges widely from 24% to 79%, showing alarmingly high rates, which could promote ESBL dissemination

TABLE 2. Geographical distribution of extended-spectrum β-lactamase (ESBL) types from colonized patients

Areas and countries	ESBL-producing *Escherichia coli*	ESBL							
		CTX-M group 1		CTX-M group 9			SHV		
		CTX-M-15	CTX-M-3	CTX-M-14	CTX-M-9	CTX-M-27	SHV-5	SHV-12	SHV-27
North Africa	6								
Egypt	3	2				1			
Morocco	3	1	1				1		
East Africa	0								
West Africa	10								
Burkina Faso	1	1							
Ghana	2	2							
Mali	2	2							
Nigeria	1							1	
Senegal	4	3[b]				1		1[b]	
Central Africa	3								
Cameroon	1	1							
Congo	1	1							
Equatorial Guinea	1					1			
South Africa	2								
Mozambique	2	1			1				
Central America	5								
Guatemala	3	2					1		
Mexico	2	1[c]		2[c]					
South America	4								
Peru	3	3							
Venezuela	1			1					
Caribbean	0								
Eastern Asia	0								
South Asia	64								
India	62	57[d]		2			1	2	1[d]
Nepal	2	2							
South East Asia	3								
Indonesia	1	1							
Malaysia	1							1	
Myanmar	1			1					
Thailand	0								
More than one region[a]	0								
Total	97	80	1	6	1	3	3	5	1

[a]South Africa and South Asia.
[b]One isolate harboured CTX-M-15 + SHV-12.
[c]One isolate harboured CTX-M-15 + CTX-M-14.
[d]One isolate harboured CTX-M-15 + SHV-27.

worldwide [16,17]. The distribution of the prevalence and type of the ESBLs isolated from colonized patients by geographical areas is shown in Tables 1 and 2.

In conclusion, among the few studies reporting the prevalence of ESBL faecal carriage in patients with travellers' diarrhoea, to our knowledge this is the first study conducted in Spain. A significant population including 457 patients was studied. ESBL-producing *E. coli* isolates were found in 17.9% of the patients returning from tropical and subtropical countries. As described previously, the most prevalent enzyme was CTX-M-15 [7–9], thereby emphasizing its dissemination worldwide [15]. Moreover, patients returning from India showed the highest prevalence of colonization (37.4%). In this study, the differences found in travel destinations and prevalences may reflect different international travel patterns for the Spanish population.

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Transparency Declaration

The authors declare no conflicts of interest.

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